Analytical characterisation of arsenic anticoccidials and their determination in poultry feed.

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(2006-109 – Drug analysis – May 2006 - NAMUR)

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Introduction

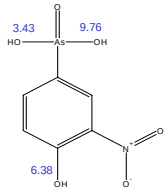


Fig.1: Roxarsone

Roxarsone (Fig.1) and nitarsone (Fig.2) are organoarsenic compounds, extensively used as feed-additive in the broiler poultry industry to control coccidial intestinal parasites and for increased rate of weight, improved feed efficiency and pigmentation. However, relatively little is known about their analytical physico-chemical characteristics. Moreover, their determination in premixes and medicated feed, spanning a wide concentration range of 20% (m/m) to as low as 50 ppm, is not well developed, increasing the risk of inappropriate dosing due to *i.a.* demixing.

In this study, the molecular properties of roxarsone were calculated and/or obtained using different commercially available programs and databases. UV-VIS spectra were recorded at different pH values, allowing to assess the pKa values and the pH species distribution. An isocratic HPLC-UV method was developed and validated, with emphasis towards robustness. Last, sample treatment was explored using solid-phase extraction (C18, C8, Phenyl, aminopropyl, SAX), resulting in a final methodology.

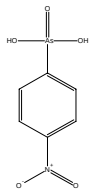


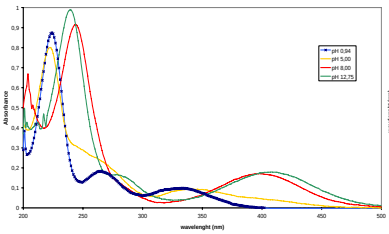
Fig.2: Nitarsone

Results and discussion

Acid-base properties of roxarsone

The pKa values of roxarsone were calculated using Advanced Chemistry Development (ACD) software and were found to be 3.43, 6.38 and 9.67 (Fig.1).

UV-VIS spectral curves at different pH values were recorded in the 300 - 500 nm range. Some characteristical curves are presented in Fig.3. The maximum absorbance between 300 and 500 nm was plotted against pH values (Fig.4). Three shifts can be observed, respecively around pH 3.75, 5.25 and 9.25, which confirmed the calculated pKa values.



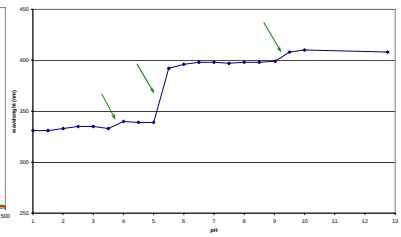


Fig.3: Roxarsone UV-VIS spectra at different pH values. **Fig.4:** Confirmation of the calculated dissociation constants.

Methodology

Different solid phase extraction (SPE) cartridges were investigated (Table1). The best results were obtained with the MaxiClean™ SPE 300 mg aminopropyl column.

FINAL METHOD

Turkey feed (label claim 20 ppm as worst case) was grinded using a laboratory blender. From these powders, 12.50 g was accurately weighted into a 100 ml Erlenmeyer flask and 50 ml 1 mM phosphate buffer (pH 5.0) was added. After centrifugation, 25 ml of the supernatant was filtered and 5 ml of the filtrate was applied onto a preconditioned MaxiClean™ SPE 300 mg aminopropyl SPE column. After washing with phosphate buffer (1 mM, pH 5.0), roxarsone was eluted from the SPE column with 2 ml 50 mM phosphate buffer (pH 7.5). The eluate was diluted 1:1 with acetonitrile/acetic acid (1.5:8.5, v/v) before HPLC analysis.

Typical chromatograms are illustrated in figure 5. The chromatografic conditions were as follows:

Column:	Nucleosil 100-5 (250x4.6mm, 5 µm)
Mobile phase:	acetonitrile/acetic acid (1.5/8.5, v/v)
Flow rate:	0.6 ml/min.
Injection volume:	25 µl

SPE cartridge	Loading buffer	Retention on column (%)	Elution buffer	Recovery (%)
C18	10 mM phosphoric acid pH 2.0	10	N/A	N/A
C8	10 mM phosphoric acid pH 2.0	47	N/A	N/A
Phenyl	10 mM phosphoric acid pH 2.0	6	N/A	N/A
Aminopropyl	1 mM phosphate buffer pH 7.5	98	NaOH 0.1 M	83
			50 mM phosphate buffer pH 7.5	93
	1 mM phosphate buffer pH 5.0	100	50 mM phosphate buffer pH 7.5	92
			50 mM phosphate buffer pH 7.5 + methanol (50:50)	107
			HCl 0.1 M in methanol	0
			50 mM phosphate buffer pH 9.0 + methanol (50:50)	75
SAX	1 mM triethylamine pH 10.3	100	50 mM phosphoric acid pH 1.15	0
			50 mM phosphoric acid pH 1.15 + methanol (50:50)	0
			50 mM phosphoric acid pH 1.15 + Acetonitrile (50:50)	0

Table 1: Comparison of the different SPE cartridges used to extract and concentrate roxarsone from turkey feed.

The maximal placebo interference at the 20 ppm level was found to be 26%, which is considered acceptable. If required, possible placebo effects can be decreased by (1) subtracting the placebo value, (2) detection at 329 nm (with LOD increase x 2), (3) performing additional purification steps developed specifically for the placebo at hand.

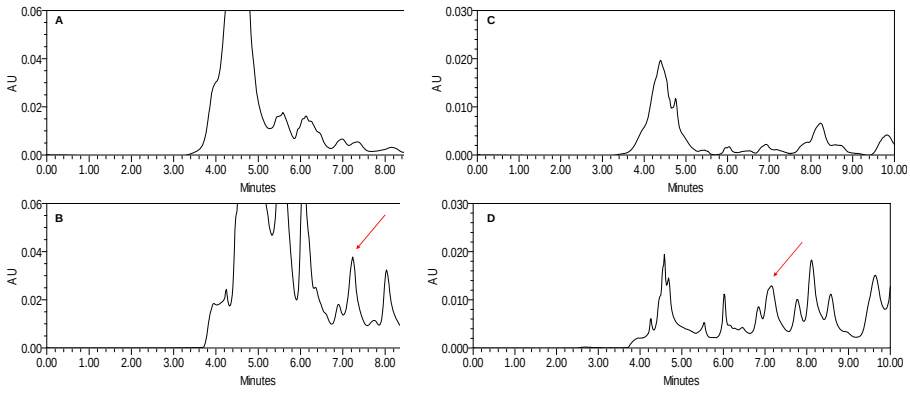


Fig.5: Roxarsone chromatograms. Placebo sample at 265 nm **(A)** and 329 nm **(C).** Sample (20 ppm) at 265 nm **(B)** and 329 nm **(D)**. The red arrow indicates roxarsone

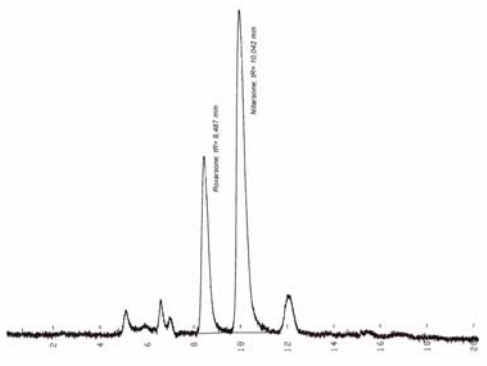


Fig.6: Illustration of the specificity of the presented HPLC method (concentration = 0.16 µg/ml both).

Conclusion

A simple and robust method for the detection and determination of roxarsone in medicated food samples as low as 20 ppm was established using the combination of an aminopropyl SPE column and RP-HPLC. This method was successfully evaluated in terms of specificity, sensitivity and robustness.